L Number	Hits	Search Text	DB	Time stamp
1	371	HS-40	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:58
3	60	HS-40 and promoter	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
2	14	HS-40 and Zeta$15	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:00
4	61	HS-40 and (promoter OR enhancer)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
5	4	(US-6303845-$).did. or (US-20020148000-$ or US-20020133838-$ or US-20020108134-$).did.	USPAT; US-PGPUB	2004/08/04 17:59
6	2	("5270184").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
9	2	("6022738").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
8	6	TCTGAGTCA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
10	8	(HS-40 OR Zeta NEAR globin).clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
11	408	HS-40 OR Zeta NEAR globin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
12	26	(HS-40 OR Zeta NEAR globin) and retrovir$5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
13	7	(US-6303845-$ or US-6022738-$ or US-6524851-$).did. or (US-20020108134-$ or US-20020148000-$ or US-20020133838-$).did. or (US-20020133838-$).did.	USPAT; US-PGPUB; DERWENT	2004/08/04 18:04

his

(FILE 'HOME' ENTERED AT 17:48:28 ON 04 AUG 2004)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 17:48:47 ON 04 AUG 2004

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L1          871 S ZETA (L) GLOBIN
L2            0 S HS-40 (L) ENAHNCER
L3          326 S HS-40
L4            0 S S1 (L) L3
L5           58 S L1 (L) L3
L6           25 DUP REM L5 (33 DUPLICATES REMOVED)
L7           15 S L6 AND PY<=1998
L8           15 SORT L7 PY
L9           10 S L6 NOT L7
L10          10 SORT L9 PY
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=> d an ti so au ab pi l10 2 5 7

L10 ANSWER 2 OF 10 MEDLINE on STN
AN 2000153760 MEDLINE
TI Loading of DNA-binding factors to an erythroid enhancer.
SO Molecular and cellular biology, (2000 Mar) 20 (6) 1993-2003.
Journal code: 8109087. ISSN: 0270-7306.
AU Wen S C; Roder K; Hu K Y; Rombel I; Gavva N R; Daftari P; Kuo Y Y; Wang C; Shen C K
AB The **HS-40** enhancer is the major cis-acting regulatory element responsible for the developmental stage- and erythroid lineage-specific expression of the human alpha-like **globin** genes, the embryonic **zeta** and the adult alpha2/alpha/1. A model has been proposed in which competitive factor binding at one of the **HS-40** motifs, 3'-NA, modulates the capability of **HS-40** to activate the embryonic **zeta-globin** promoter. Furthermore, this modulation was thought to be mediated through configurational changes of the **HS-40** enhanceosome during development. In this study, we have further investigated the molecular basis of this model. First, human erythroid K562 cells stably integrated with various **HS-40** mutants cis linked to a human alpha-**globin** promoter-growth hormone hybrid gene were analyzed by genomic footprinting and expression analysis. By the assay, we demonstrate that factors bound at different motifs of **HS-40** indeed act in concert to build a fully functional enhanceosome. Thus, modification of factor binding at a single motif could drastically change the configuration and function of the **HS-40** enhanceosome. Second, a specific 1-bp, GC-->TA mutation in the 3'-NA motif of **HS-40**, 3'-NA(II), has been shown previously to cause significant derepression of the embryonic **zeta-globin** promoter activity in erythroid cells. This derepression was hypothesized to be regulated through competitive binding of different nuclear factors, in particular AP1 and NF-E2, to the 3'-NA motif. By gel mobility shift and transient cotransfection assays, we now show that 3'-NA(II) mutation completely abolishes the binding of small MafK homodimer. Surprisingly, NF-E2 as well as AP1 can still bind to the 3'-NA(II) sequence. The association constants of both NF-E2 and AP1 are similar to their interactions with the wild-type 3'-NA motif. However, the 3'-NA(II) mutation causes an approximately twofold reduction of the binding affinity of NF-E2 factor to the 3'-NA motif. This reduction of affinity could be accounted for by a twofold-higher rate of dissociation of the NF-E2-3'-NA(II) complex. Finally, we show by chromatin immunoprecipitation experiments that only binding of NF-E2, not AP1, could be detected in vivo in K562 cells around the **HS-40** region. These data exclude a role for AP1 in the developmental regulation of the human alpha-**globin** locus via the 3'-NA motif of **HS-40** in embryonic/fetal erythroid cells. Furthermore, extrapolation of the in vitro binding studies suggests that factors other than NF-E2, such as the small Maf homodimers, are likely involved in the regulation of the **HS-40** function in vivo.

L10 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2001:757858 CAPLUS
DN 135:314417
TI Vectors containing mutated **HS-40** enhancer of . **zeta**.-**globin** gene promoter and its regulation of transgene expression in transgenic mice
SO U.S., 7 pp., Division of U.S. Ser. No. 205,015, abandoned.
CODEN: USXXAM
IN Shen, Che-Kun James
AB The invention relates to a mutated **HS-40** enhancer of . **zeta**.-**globin** gene promoter, a 350-400 bp enhancer element located about 40 kb upstream of .**zeta**.-**globin** gene. **HS-40** is the major cis-acting regulatory element responsible for the developmental stage-and erythroid lineage-specific expression of the human α-like **globin** genes, the embryonic .**zeta**. and the adult α2/α/1. The invention is based on the discovery that a single nucleotide change in the 3'NF-E2/AP1 element of the human **HS-40** enhancer, unlike the wild type **HS-40** enhancer, confers position-independent and copy number-dependent expression on a transgene. In addition, the single nucleotide change allows expression of the gene in the cells of an adult mouse, an effect not seen for the wild type **HS -40** enhancer. Accordingly, the invention provides a viral expression vector (e.g., a retrovirus) expressing a transgene regulated by (1) a transcriptional start site; (2) a promoter (e.g., a tissue-specific promoter such as .**zeta**.-**globin** promoter) operably linked to the transcriptional start site; and (3) the above mutated **HS-40** enhancer operably linked to the promoter.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6303845	B1	20011016	US 2000-536094	20000324
	US 2002133838	A1	20020919	US 2001-961563	20010920
	US 2002108134	A1	20020808	US 2001-977432	20011015
	US 2002148000	A1	20021010	US 2001-14220	20011109

L10 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2003:1004738 CAPLUS
DN 140:1576
TI A strong variant of the HS-40 enhancer and its use in expression vectors for transgenic animals
SO U.S. Pat. Appl. Publ., 13 pp., Cont.-in-part of U.S. Ser. No. 961,563.
CODEN: USXXCO
IN Shen, Che-kun James
AB A substitution mutant of the **HS-40** enhancer of . **zeta**.-**globin** gene promoter, a 350-400 bp enhancer element located about 40 kb upstream of .**zeta**.-**globin** gene is used in expression vectors for high level expression of foreign genes in transgenic animals. **HS-40** is the major cis-acting regulatory element responsible for the developmental stage-and erythroid lineage-specific expression of the human α-like **globin** genes, the embryonic .**zeta**. and the adult α2/α/1. A single nucleotide change in the 3'NF-E2/AP1 element of the human **HS-40** enhancer, unlike the wild type **HS-40** enhancer, confers position-independent and copy number-dependent expression on a transgene. The mutation also relieves the developmental regulation of expression from the promoter of the . **zeta**.-**globin** gene. In addition, the single nucleotide change allows expression of the gene in the cells of an adult mouse, an effect not seen for the wild type **HS-40** enhancer. The transgenic animal may include pig, rat, cow, rabbit, goat, guinea pig, prairie baboon, squirrel, monkey, chimpanzee, bird, frog, toad, chicken, turkey and sheep. The generation of transgenic mice expressing a growth hormone gene in erythroblasts using the **HS-40**(mt) enhancer and the .**zeta**.-**globin** promoter is demonstrated. Serum growth hormone levels of up to 6,490 ng/mL were obtained.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002148000	A1	20021010	US 2001-14220	20011109
	US 6303845	B1	20011016	US 2000-536094	20000324

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(FILE 'HOME' ENTERED AT 17:48:28 ON 04 AUG 2004)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 17:48:47 ON 04 AUG 2004

L1 871 S ZETA (L) GLOBIN
L2 0 S HS-40 (L) ENAHNCER
L3 326 S HS-40
L4 0 S S1 (L) L3
L5 58 S L1 (L) L3
L6 25 DUP REM L5 (33 DUPLICATES REMOVED)
L7 15 S L6 AND PY<=1998
L8 15 SORT L7 PY

=> d an ti so au ab pi 18 1 5 7 9 10

L8 ANSWER 1 OF 15 MEDLINE on STN
AN 91342671 MEDLINE
TI Characterization of the major regulatory element upstream of the human alpha-globin gene cluster.
SO Molecular and cellular biology, **(1991 Sep)** 11 (9) 4679-89.
Journal code: 8109087. ISSN: 0270-7306.
AU Jarman A P; Wood W G; Sharpe J A; Gourdon G; Ayyub H; Higgs D R
AB The major positive regulatory activity of the human alpha-**globin** gene complex has been localized to an element associated with a strong erythroid-specific DNase I hypersensitive site (**HS -40**) located 40 kb upstream of the **zeta** 2-**globin** mRNA cap site. Footprint and gel shift analyses of the element have demonstrated the presence of four binding sites for the nuclear factor GATA-1 and two sites corresponding to the AP-1 consensus binding sequence. This region resembles one of the major elements of the beta-**globin** locus control region in its constitution and characteristics; this together with evidence from expression studies suggests that **HS -40** is a primary element controlling alpha-**globin** gene expression.

L8 ANSWER 5 OF 15 MEDLINE on STN
AN 93204975 MEDLINE
TI Transcriptional activation of human **zeta** 2 **globin** promoter by the alpha **globin** regulatory element (**HS-40**): functional role of specific nuclear factor-DNA complexes.
SO Molecular and cellular biology, **(1993 Apr)** 13 (4) 2298-308.
Journal code: 8109087. ISSN: 0270-7306.
AU Zhang Q; Reddy P M; Yu C Y; Bastiani C; Higgs D; Stamatoyannopoulos G; Papayannopoulou T; Shen C K
AB We studied the functional interaction between human embryonic **zeta** 2 **globin** promoter and the alpha **globin** regulatory element (**HS-40**) located 40 kb upstream of the **zeta** 2 **globin** gene. It was shown by transient expression assay that **HS-40** behaved as an authentic enhancer for high-level **zeta** 2 **globin** promoter activity in K562 cells, an erythroid cell line of embryonic and/or fetal origin. Although sequences located between -559 and -88 of the **zeta** 2 **globin** gene were dispensable for its expression on enhancerless plasmids, they were required for the **HS-40** enhancer-mediated activity of the **zeta** 2 **globin** promoter. Site-directed mutagenesis demonstrated that this **HS-40** enhancer-**zeta** 2 **globin** promoter interaction is mediated by the two GATA-1 factor binding motifs located at -230 and -104, respectively. The functional domains of **HS-40** were also mapped. Bal 31 deletion mapping data suggested that one GATA-1 motif, one GT motif, and two NF-E2/AP1 motifs together formed the functional core of **HS-40** in the erythroid-specific activation of the **zeta** 2 **globin** promoter. Site-directed mutagenesis further demonstrated that the enhancer function of one of the two NF-E2/AP1 motifs of **HS-40** is mediated through its binding to NF-E2 but not AP1 transcription factor. Finally, we did genomic footprinting of the **HS-40** enhancer region in K562 cells, adult nucleated

erythroblasts, and different nonerythroid cells. All sequence motifs within the functional core of **HS-40**, as mapped by transient expression analysis, appeared to bind a nuclear factor(s) in living K562 cells but not in nonerythroid cells. On the other hand, only one of the apparently nonfunctional sequence motifs was bound with factors in vivo. In comparison to K562, nucleated erythroblasts from adult human bone marrow exhibited a similar but nonidentical pattern of nuclear factor binding in vivo at the **HS-40** region. These data suggest that transcriptional activation of human embryonic **zeta** 2 **globin** gene and the fetal/adult alpha **globin** genes is mediated by erythroid cell-specific and developmental stage-specific nuclear factor-DNA complexes which form at the enhancer (**HS-40**) and the **globin** promoters.

L8 ANSWER 7 OF 15 MEDLINE on STN
AN 95327665 MEDLINE
TI Transcriptional activation of human adult alpha-globin genes by hypersensitive site-40 enhancer: function of nuclear factor-binding motifs occupied in erythroid cells.
SO Proceedings of the National Academy of Sciences of the United States of America, **(1995 Jul 3)** 92 (14) 6454-8.
Journal code: 7505876. ISSN: 0027-8424.
AU Rombel I; Hu K Y; Zhang Q; Papayannopoulou T; Stamatoyannopoulos G; Shen C K
AB The developmental stage- and erythroid lineage-specific activation of the human embryonic **zeta**- and fetal/adult alpha-**globin** genes is controlled by an upstream regulatory element [hypersensitive site (**HS**)-**40**] with locus control region properties, a process mediated by multiple nuclear factor-DNA complexes. In vitro DNase I protection experiments of the two G+C-rich, adult alpha-**globin** promoters have revealed a number of binding sites for nuclear factors that are common to HeLa and K-562 extracts. However, genomic footprinting analysis has demonstrated that only a subset of these sites, clustered between -130 and +1, is occupied in an erythroid tissue-specific manner. The function of these in vivo-occupied motifs of the alpha-**globin** promoters, as well as those previously mapped in the **HS-40** region, is assayed by site-directed mutagenesis and transient expression in embryonic/fetal erythroid K-562 cells. These studies, together with our expression data on the human embryonic **zeta-globin** promoter, provide a comprehensive view of the functional roles of individual nuclear factor-DNA complexes in the final stages of transcriptional activation of the human alpha-like **globin** promoters by the **HS-40** element.

L8 ANSWER 9 OF 15 MEDLINE on STN
AN 95238333 MEDLINE
TI Functional roles of in vivo footprinted DNA motifs within an alpha-globin enhancer. Erythroid lineage and developmental stage specificities.
SO Journal of biological chemistry, **(1995 Apr 14)** 270 (15) 8501-5.
Journal code: 2985121R. ISSN: 0021-9258.
AU Zhang Q; Rombel I; Reddy G N; Gang J B; Shen C K
AB Transcriptional regulation of the human alpha-like **globin** genes, embryonic **zeta** 2 and adult alpha, during erythroid development is mediated by a distal enhancer, **HS-40**. Previous protein-DNA binding studies have shown that **HS-40** consists of multiple nuclear factor binding motifs that are occupied in vivo in an erythroid lineage- and developmental stage-specific manner. We have systematically analyzed the functional roles of these factor binding motifs of **HS-40** by site-directed mutagenesis and transient expression assay in erythroid cell cultures. Three of these **HS-40** enhancer motifs, 5'NF-E2/AP1, GT II, and GATA-1(c), positively regulate the **zeta** 2-**globin** promoter activity in embryonic/fetal erythroid K562 cells and the adult alpha-**globin** promoter activity in adult erythroid MEL cells. On the other hand, the 3'NF-E2/AP1 motif is able to exert both positive and negative regulatory effects on the **zeta** 2-**globin** promoter activity in K562 cells, and this dual function appears to be modulated through differential binding of the ubiquitous AP1 factors and the erythroid-enriched NF-E2 factor. Mutation in the GATA-1(d) motif,

which exhibits an adult erythroid-specific genomic footprint, decreases the **HS-40** enhancer function in dimethyl sulfoxide-induced MEL cells but not in K562 cells. These studies have defined the regulatory roles of the different **HS-40** motifs. The remarkable correlation between genomic footprinting data and the mutagenesis results also suggests that the erythroid lineage- and developmental stage-specific regulation of human alpha-like **globin** promoters is indeed modulated by stable binding of specific nuclear factors in vivo.

L8 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1996:128940 CAPLUS
DN 124:166363
TI Transcriptional regulation of human ζ2 and α globin promoters by multiple nuclear factor-DNA complexes: The final act
SO Molecular Biology of Hemoglobin Switching, Proceedings of the Conference on Hemoglobin Switching, 9th, Orcas Island, Wash., June 10-14, 1994 (**1995**), Meeting Date 1994, 193-202. Editor(s): Stamatoyannopoulos, George. Publisher: Intercept, Andover, UK.
CODEN: 62JIAN
AU Zhang, Qingyi; Rombel, Irene; Reddy, G. Narender; Shen, C. -K. James
AB A review, with 29 refs. Site-directed mutagenesis and transient expression assay were used to analyze functional contributions of individual nuclear factor-binding motifs to the transcriptional regulation of the two human α-like **globin** promoters, embryonic . **zeta**.2, and adult α, by the **HS-40** element in embryonic/fetal erythroid K562 cells and adult erythroid MEL cells.

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